

THE PERSISTENCE OF BACTERIA IN THE MILK DUCTS OF THE COW'S UDDER.

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The constant presence of bacteria in freshly drawn milk is a matter of considerable importance. The fact that milk when drawn from the udder may contain bacteria is of the greatest interest in connection with the observance of measures designed to reduce the bacterial content of milk to the minimum. Here, if that fact be true, is one source of the infection of milk which can not be eliminated by the exercise of precautions during the milking or the subsequent processes to which it is subjected.

The earlier investigations undertaken to throw light on the question of the presence of bacteria within the healthy udder consisted in counting the bacteria in samples taken during different periods of the milking. Schultz* found a decrease in numbers as the milking progressed. Lehmann† obtained like results. It might be concluded from the work of Schultz and Lehmann that the teats, or at most the lower portion of the cistern, only contain certain bacteria.

Gernhardt‡ found a larger number in samples from the middle of the milking than at the beginning, although some of the samples from the last milk drawn were sterile. To explain his results, Gernhardt suggests that the bacteria make their way up through the milk ducts of the teats, through the cistern and into the smaller ramifications of the ducts which connect the cistern with the ultimate follicles. Such an assumption explains the wide variation in numbers obtained by him.

* Leopold Schultz. *Archiv. f. Hygiene*, B. S. XIV. (1892).

† Lehmann. *17te Versammlung d. deut. Ver. f. öffent. Gesundheitspflege*.

‡ Gernhardt. *Quant. Spaltpilzunters. d. Milch*, Inaug. Dissert. Univ. Jurjew.

E. von Freudenreich* states that when in the udder, milk is free from bacteria except when the milk glands are in a diseased condition. He mentions the fact as having been demonstrated by Pasteur who drew samples directly from the cistern by means of a sterile cannula. On the other hand, Bolley and Hall† compared the species of bacteria in the milk of several cows, the samples being taken through a sterile milking tube inserted into the milk cistern.

Russell‡ found that bacteria are present in the udder proper in case of mastitis. In Russell's Dairy Bacteriology we find the following: "How far these different forms of germ life are able to penetrate into the healthy udder is as yet unknown. In all probability, the glandular tissue of the udder is not affected, although it is possible that microbes might work their way up the open channel of the teat into the udder proper".

Grotenfelt§ says: "When the milk is drawn from the udder of a healthy cow it is germ free, or sterile. The original sterility of normal milk is due to the fact that the bacteria can not gain access to the milk glands from without as long as the udder is not injured in any way". F. W. Woll, the translator of Grotenfelt's work, adds in a foot note: "The bacteria in the milk cistern will be mostly washed out by the first milk drawn, but not all removed until milking has progressed some time".

Rotch|| concludes that the few cases in which contaminated samples were obtained from the strippings, were due to faults in technique and not to bacteria from the interior of the udder.

*Ed. von Freudenreich. Dairy Bacteriology (1895), translated by J. R. A. Davis. Page 36.

†Bolley and Hall. Abstract in Experiment Station Record, Vol. VII., No. 11, p. 991.

‡H. L. Russell. Dairy Bacteriology, 2nd edition, pp. 42, 43.

§Gösta Grotenfelt. The Principles of Modern Dairy Practice, translated by F. W. Woll. Page 23.

||T. M. Rotch. Transactions of the Association of American Physicians (1894).

Moore* reviews the conclusions of Schultz, Gernhardt and Rotch and gives the results of his own investigations. In every examination made, he found the last milk from at least one quarter of the udder to contain bacteria. In concluding his paper, Moore suggests that a bacteriologic examination of the larger milk ducts and of the acini themselves might throw some light upon the assumption of Gernhardt. Such an investigation was rendered impossible at the time on account of his inability to procure the udder of a freshly killed milch cow.

That sterile samples may frequently be obtained directly from the teat is a fact that has been demonstrated by many investigators. But the frequency with which these same workers have failed, leads to the conclusion that the last milk contains only a few bacteria and which may or may not be contained in a given small sample. Schultz, Gernhardt, Russell, Rotch and Moore have all been unable to get sterile milk in every case. Information is not at hand concerning the amount of milk taken for a sample, except that Moore took 50 cc. of the last milk. Conn† suggests that the reason the earlier workers obtained sterile milk so readily was because they did not collect large samples. He says "Essentially the same facts have been demonstrated in regard to human milk. * * * Honigmann¹, Knochenstiern², Ringel³, and Palleske⁴, have all independently found that it is impossible to get human milk fresh from the mammary gland in such a way as to be sterile".

* V. A. Moore. Preliminary Investigations concerning the number and nature of Bacteria in Freshly Drawn Milk. Twelfth and Thirteenth Annual Reports of the Bureau of Animal Industry U. S. Dept. Agr. p. 261.

† W. H. Conn. Bull. No. 25, U. S. Department of Agriculture, Office of Experiment Stations.

1. Honigmann. Ztscher. Hyg. 14 (1893), p. 207.
2. Knochenstiern. Inaug. Diss. Dorpat, (1893).
3. Ringel. Münch. Med. Wochenschr., (1893), p. 513.
4. Palleske. Virch. Arch., 130 (1892), p. 135.

E. von Freudenreich* states that he failed to obtain sterile milk in large quantities although the udder was washed and smeared with lard, to prevent contamination. In an attempt to collect ten liters of sterile milk for an experiment in cheese making, he was unable to reduce the number below 212 bacteria per cubic centimeter. He calls attention to the ease with which a few cubic centimeters are collected, using the same precautions, but he does not recognize the presence of bacteria from within the udder.

Those who believe the last milk as drawn from the teat to be absolutely sterile, must necessarily explain the constant presence of bacteria in the fore milk. The explanation is substantially as follows: Bacteria in the air or in stable filth accidentally gain a foothold in the milk remaining on the end of the teat after milking. The favorable conditions for bacterial growth offered by the ducts favor the multiplication of the invading bacteria which increase so rapidly as to account for the presence of the multitudes always found in the fore milk. Experiments by the writer have shown that it is possible for this to occur under certain conditions, but the more probable explanation is embodied in the results of the investigations about to be described. These will be treated under three separate heads, as they have in common only the fact that they lead to the same conclusion.

THE PERSISTENCE OF CERTAIN SPECIES OF BACTERIA IN THE FORE MILK.

The work of Bolley and Hall is the only investigation on the subject that has come to notice. Samples of milk were taken by means of a sterile milking tube inserted through the duct into the milk cistern. Some species were found common to both the first and the last milk drawn. Only one organism was found common to the milk of all the animals examined, that one having no effect upon the milk. The writers conclude that a given form, once present, may be quite constant in its occupancy of the udder in an individual.

* Ed. von Freudenreich. *Landwirtschaftliches Jahrbuch der Schweiz*. 1890, II., p. 18.

In the investigations which I have made to determine the nature of the milk duct flora, the following methods were followed: Before collecting samples, the udder and flanks of the cow were thoroughly moistened to prevent the dislodgment of dust by the movements of milking. In addition, the teats were moistened with a solution of mercuric chloride. Samples were drawn directly from the teat into sterile test tubes which were provided with cotton plugs. In this respect the work of the writer differs from that of Bolley and Hall. Cultures were made immediately after collecting the samples. Five two hundred and fiftieths (5-250) of a cubic centimeter of milk was found, in general, to introduce a sufficient number of bacteria for convenient study upon a plate culture made in 15 cc. of medium. For a time both gelatin and agar plate cultures were made, but the use of the former was discontinued, as agar was found to be more satisfactory. The total number of colonies did not appear until after several days in the incubator at a temperature of 37.5° C. The plates were then carefully examined and sub-cultures were made from the colonies of the apparently different species. The various forms of colonies were carefully described and the number of each recorded.

The milk of each of the four teats of the cow was examined on two successive days and after a lapse of two weeks, some of them were examined upon four more days. Four or possibly five species were observed, only one being common to the four teats. Although the bacterial flora of each of the teats differed from that of its neighbors, the same species were found to persist in the same teat from day to day. They were not present in the same relative numbers on each occasion.

The milk of another cow was examined on five occasions covering a period of eight months. In the milk of this animal but three species of bacteria were found. On the first day that the milk was examined, a streptococcus was found to predominate in numbers in all four of the teats. The other two species occurred only occasionally, but in the later examinations they were found to exceed the streptococcus in number.

The presence of streptococci in milk from a normal udder is,

in the experience of the writer, unusual. None have been found in the milk of eight other cows in the same stable, or for that matter, in any examination of fore milk from cows elsewhere. The persistence of the streptococcus in the milk of the one cow is therefore of special significance.

The mathematical probability that the same organism will invade the same sterile milk duct, even twice in succession, is infinitely slight. It is therefore necessary to seek other explanation for the constant presence of bacteria in the fore milk, when we consider the persistence of species in the milk of certain cows or in particular quarters of the udder of the same cow.

AN EXPERIMENT IN COLONIZING THE CISTERN WITH BACTERIA.

With reference to determining the possibility for an organism to persist in the cistern for a considerable period, it was determined to introduce into one quarter of the udder, a culture of an easily distinguished bacillus. For this purpose *Bacillus prodigiosus* was selected because the red color of its growth on agar would render its presence in milk easily recognized when cultures were made. Four cubic centimeters of a bouillon culture were introduced into the cistern by means of a hyperdermic syringe lengthened with a milking tube. Both the milking tube and the syringe were scalded to guard against introducing any other micro-organisms along with *prodigiosus*. It was known from work already done that the organism in question was not a natural inhabitant of the udder upon which the experiment was being made.

The use of the milking tube as is nearly always the case, occasioned an inflammation of one side of the udder. The inflammation is attributed to the use of the milking tube rather than to *Bacillus prodigiosus*. The threatened obstruction of the teat by the accumulation of irregularly shaped masses of casein, rendered it necessary to frequently draw out the purulent liquid from the diseased quarter of the udder during the two following days after which the inflammatory condition subsided. Plate cultures were made each day. On the day following the inoculation, ten colonies of *Bacillus prodigiosus*

appeared on the plates. Although the same amount of milk was used in making the cultures on the days following, the number of colonies was observed to decrease in number. On the sixth day, the colonies of that bacillus ceased to appear. During the whole period, with the exception of the first two days, colonies of the native bacterial flora were observed in each plate culture.

The fact that an organism selected at random, without considering its fitness for inhabiting the udder, should succeed in persisting there for six days is significant. The experiment demonstrates the fact that frequent and thorough milkings may not remove all bacteria from the udder. That other species of bacteria, better fitted for that environment are able to persist in the udder for longer periods, seems highly probable.

A BACTERIOLOGIC EXAMINATION OF THE GLANDULAR TISSUE OF THE UDDER.

The writer is indebted to Dr. Moore for the suggestion of this line of work and for the privilege of associating with him in an investigation based upon it. A partial report of the results obtained has been published elsewhere*. In attempting to draw conclusions from the facts which have already been presented, the writer finds himself unavoidably influenced by the facts brought to light in the work to which reference has been made. That his conclusions may not appear to be based upon a less firm foundation of fact than is the case, he feels justified in here referring to the joint labor.

The fundamental method underlying the investigation consisted in making a large number of cultures directly from freshly exposed glandular tissue. Sterile tubes, containing about 15 cc. each of gelatin, and some containing slanted agar were taken to the place of slaughter.

The purpose was to compare the bacteria found in the fore milk with those which might be found in the udder itself.

*V. A. Moore and A. R. Ward. Bull. No. 158, Cornell University Agricultural Experiment Station.

Samples of the fore milk and in one case, of the strippings, were taken immediately before the slaughter. In order to obtain more definite results, each quarter of the udder was arbitrarily divided into three divisions. The first (A, Pl. V.) included the teat and milk cistern. The second and third divisions (B, C) included horizontal zones of equal thickness constituting the remaining portion of the udder.

Immediately after slaughtering the cow, the udder was carefully removed. The skin was reflected and a flamed knife was used to make a dorso-ventral incision several inches in depth in one quarter of the udder. Samples of milk were collected in sterile test tubes as it welled out of the cistern and its smaller ramifications. In making cultures from the glandular tissue, care was taken to prevent milk of the ventral region from coming in contact with the freshly exposed surfaces. Bits of tissue were detached with flamed scissors, and transferred to culture media by the use of a flamed platinum loop. Tubes of gelatin and of agar were inoculated in this manner from each of the three arbitrarily designated divisions of the quarter. The same procedure was repeated with each of the other three quarters of the udder. Cultures were made from the udders of six cows in the manner described.

Upon returning to the laboratory, the gelatin was liquified at a temperature not exceeding 37° C. and poured into sterile Petri dishes where it again became solid. Agar plate cultures were made from the milk samples, and together with those slanted agar cultures already inoculated, were placed in the incubator. The agar plate cultures were designed to be used as a check upon the reliability of the conclusions reached from an examination of the other cultures. For instance: It might be possible that organisms appearing to have been obtained from the interior of the udder, may have lodged upon the bits of tissue during the transfer. The identity in cultural and morphologic characters of bacteria found in cultures made from the fore milk and the glandular tissue of the udder would eliminate a source for false conclusions.

The tubes of slanted agar after standing in the incubator for several days, were examined particularly with reference to the presence or absence of growth. Nearly all of the media which had been in contact with material from the udder, showed growth. Note was taken of the color and character of the growth of the colonies and sub-cultures were made.

The gelatin plate cultures were in like manner examined, furnishing a more satisfactory method for obtaining pure cultures. With these, a direct comparison made it possible to trace the presence of the same organism in the three localities. In order to prove that these identities existed, sub-cultures were made for a more detailed comparison later. The plate cultures made from the milk were examined and sub-cultures were made from all of the apparently different colonies.

By comparing cultures from the various sources, it was found that the same organism frequently occurred in the fore milk and in each of the three parts of the udder. Most of the bacteria obtained in pure cultures were found to belong to one of three species of micrococci. Cultures of the three species were obtained from a sufficient variety of sources to demonstrate their general distribution throughout the udder.

The apparently healthy udders of six milch cows were in that manner found to contain bacteria in the depths of the milk-secreting tissue. By the methods employed, it was impossible to detect any difference in the relative numbers of bacteria present in the three regions of the udder.

The evidence at hand indicates that the teats and the greater portion of the udder may normally contain bacteria. It also seems highly probable that a few at least of the organisms found in the udder remain there after each milking, becoming the progenitors of the organisms found to be present in the milk when drawn. This conclusion seems to be supported by the following facts.

1. Certain species of bacteria have been found to persist in particular quarters of the udder for considerable periods of time. This controverts the statement that the milk ducts are sterile at the close of the milking, becoming tenanted from

the outside by any organisms which by chance come in contact with the end of the duct.

2. It is possible for bacteria to remain in the udder and not be ejected along with the milk. This has been proven possible in the case of one organism. A culture of *Bacillus prodigiosus* has been introduced into the milk cistern and has succeeded in persisting there for six days, as was shown by its presence for that period in the milk of that quarter of the udder.

3. Cultures of bacteria have been obtained by Dr. Moore and the writer from the glandular tissue of the udders of freshly killed milch cows. Identical species of micrococci were obtained from the milk and from the glandular tissue of the udder.

4. It has not been shown by the investigations published up to this time that the last milk drawn is always sterile.

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PLATE V.

A photograph of a section through the teat and one quarter of the udder of a cow. The parts represented by the letters *A*, *B*, *C*, indicate the three arbitrary divisions into which the gland was divided for purposes of examination.

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